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RE: Summary of PFAS Treatment, Monitoring and Findings at Truax Field – Building 430 – Madison WI

Introduction

The following is a summary of the pilot scale PFAS treatment conducted for the Wisconsin Air National Guard and Dane County Regional Airport at Truax Field. The work began in May 2021 and extended through November 2022. Over this time two primary remedial activities took place; an ex-situ treatment involving PFAS impacted fire engine washout and an in-situ DPT remedial injection. This report details the treatment methods, findings, and analysis of the work completed by ORIN Technologies and Fixed Earth.

Site Setting and Condition

The study area is located at Truax Field in Madison, WI. In particular, the site is located to the southwest of Building 430. This area has been documented to be historically used for firefighting nozzle testing and was identified as an area of potential environmental concern as it related to contamination from poly- and perfluoroalkyl substances (PFAS) which were used extensively in airport firefighting efforts until recent years.

The study area itself consists of an approximately 1600 square foot area that is grass covered and level. It is adjacent to active runways and roadways used in the operation of the base and Dane County Regional Airport. Airport surface drainage infrastructure can be found to the east and southeast of the study area, with site surface drainage generally being carried to the south. Figure 1 provides a general overview of the study area. Figure 1A shows the well and electrode layout.

Figure 1. Aerial overview of study area.

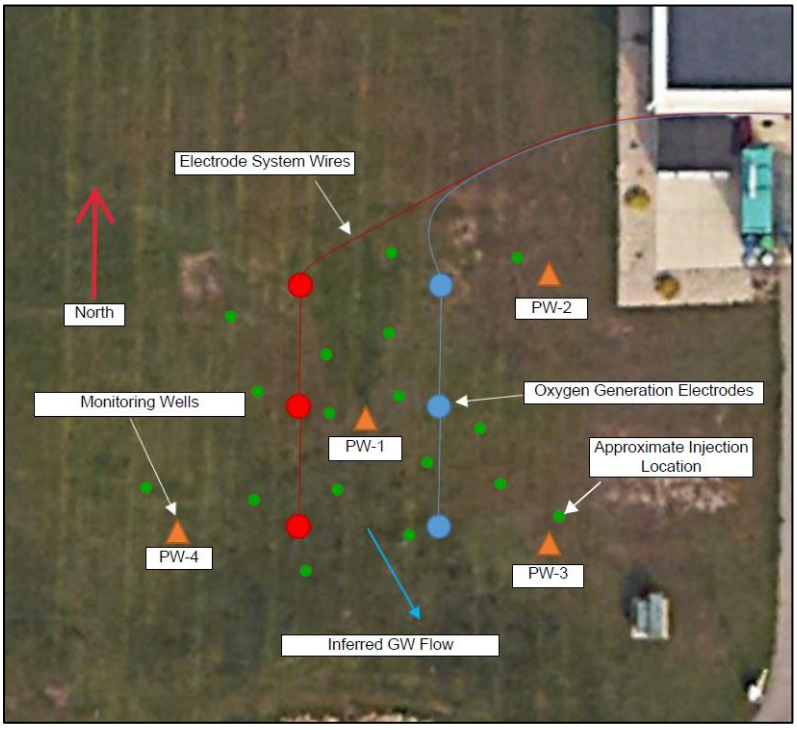

Figure 1A. Site layout overview. Locations are approximate.

The in-situ study discussed in this report included the surface area described above and was completed to a depth of approximately 25 ft below ground surface, encompassing a soil volume of 1200 cubic meters (the treatment block). The soils throughout the treatment block were found to consist of fine sand. The study also included groundwater that fluxed through the treatment block throughout the duration of the study. Groundwater conditions and flux are described in later sections of this report.

Summary of Historical Studies

Prior to the commencement of the in-situ field study discussed in this report, a lab study to isolate and validate site-specific microorganisms was completed by Fixed Earth in early 2021. This report has been included as Appendix C to this report. In summary, four microbial isolates were collected from the subject location, 3 bacterial and 1 fungal, that were capable of surviving in PFAS as the sole carbon source in the growth media. Additionally, these microbial isolates were found to form fluoride ions when grown in the presence of PFAS, as indicated by a colorimetric assay. Lab analysis of water samples spiked with known quantities of PFAS that were treated with these microbial isolates showed a significant decline in PFAS concentrations.

Later in 2021 an ex-situ field study commenced in which four 1000 L totes containing PFAS concentrates ranging from 7 to 33 ppm total PFAS were treated at the airport's facilities. Three of the totes (Totes 1, 3, and 4) were treated using a mixture of BAM, site-specific bacteria, calcium peroxide for an initial oxygen boost, and aeration to maintain aerobic conditions. Tote 2 was treated with site-specific bacteria and aeration alone to demonstrate the potential of the bacteria to degrade PFAS and to eliminate concerns that the sorption to BAM was responsible for PFAS disappearance alone.

Totes treated using a combination of BAM and microbes achieved a 90% reduction in total PFAS concentrations within 5 weeks of treatment and had achieved greater than 99% total PFAS removals within 15 weeks of treatment. No rebound of PFAS concentrations was observed in the subsequent 20 weeks of data collection. Tote 2, treated with microbes and aeration alone, achieved 90% total PFAS removal 15 weeks after treatment. A significant spike in total PFAS concentrations was observed in Tote 2 prior to the reductions and is most likely attributed to precursor transformation under aerobic conditions. Tote 2 ultimately achieved 95% reductions in total PFAS and no rebound occurred. Figure 2 provides an overview of total PFAS removals in each tote.

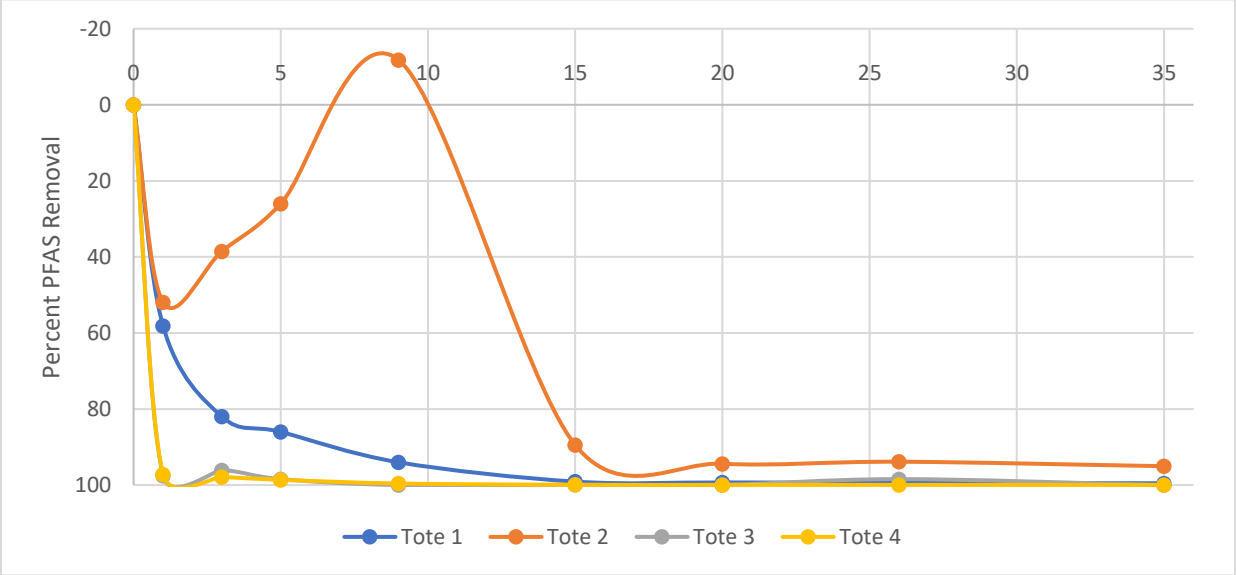

Figure 2. Summary of ex-situ tote treatability study showing total PFAS removals as measured by EPA 537.

As in previous lab studies completed internally, for The State of Michigan, and for this site, the generation of short chain carboxylic acids, primarily PFBA and PFPeA, was observed in Tote 1 and 2. This generation of short chain carboxylates is a general indicator of biological activity against longer chain PFAS.

Sorption of PFAS species to bacterial cell membranes is a common concern brought up in relation to this study. Using PFAS sorption values calculated by Butzen et al (2020), it was determined that approximately 317 lbs of cell mass would have been required to sorb the 12 ppm of PFAS found in Tote 2 to the extent observed. This quantity of biomass was not observed in Tote 2 at the conclusion of the study. Another common concern with PFAS treatability studies is the tendency for PFAS to partition within tanks of water, resulting in areas of higher and lower concentrations. To address these concerns, a defoaming agent was added to each tote to minimalize foam fractionation and each tank was agitated with a dedicated paddle mixer prior to sampling to homogenise the fluid.

Groundwater Physiochemical Conditions and Geology

Shallow groundwater at the site was found to range between 6.25 and 8.25 ft below ground surface (bgs) throughout the duration of the study period. Groundwater elevations reached their annual minimum on the February 23, 2022 monitoring event, with groundwater depths averaging approximately 8 ft bgs. During spring freshet groundwater depths were found to rise steeply, rising approximately 1.5 ft throughout March 2021. A period of sustained moisture occurred following freshet which maintained groundwater depths at these elevated positions until mid-May when they started to decline.

Historical studies of groundwater flow at Truax Field have shown that groundwater flow direction can change over the course of a year and can vary depending on localized conditions, such as historical

subsurface infrastructure. However, the Relative Risk Site Evaluation completed for the base indicates that groundwater flow is generally towards the south and southeast. Groundwater elevations within the study area are in general agreement with this finding and indicated groundwater movement to the south-southeast throughout the duration of the study. Groundwater movement throughout the study area may also be influenced by buried infrastructure occurring in the project area as well as surface drainage ditches to the southeast of the study area that may act as discharge points.

Multiple large rainfall events occurred throughout the study period which contributed to spikes in groundwater elevation and were potentially related to vadose zone flushing of PFAS at multiple intervals throughout the study. Particularly notable rainfall events include: April 22 (0.92 in), May 25/26 (1.74 in), June 8 (0.85 in), June 15 (1.37 in), and July 4/5 (2.68 in). In general, it was found that groundwater elevations in the study area responded rapidly to rainfall events, with an increase in groundwater elevation typically observed within one week of a significant rainfall event. Figure 3 provides an overview of groundwater elevations at each of the four monitoring points throughout the project duration.

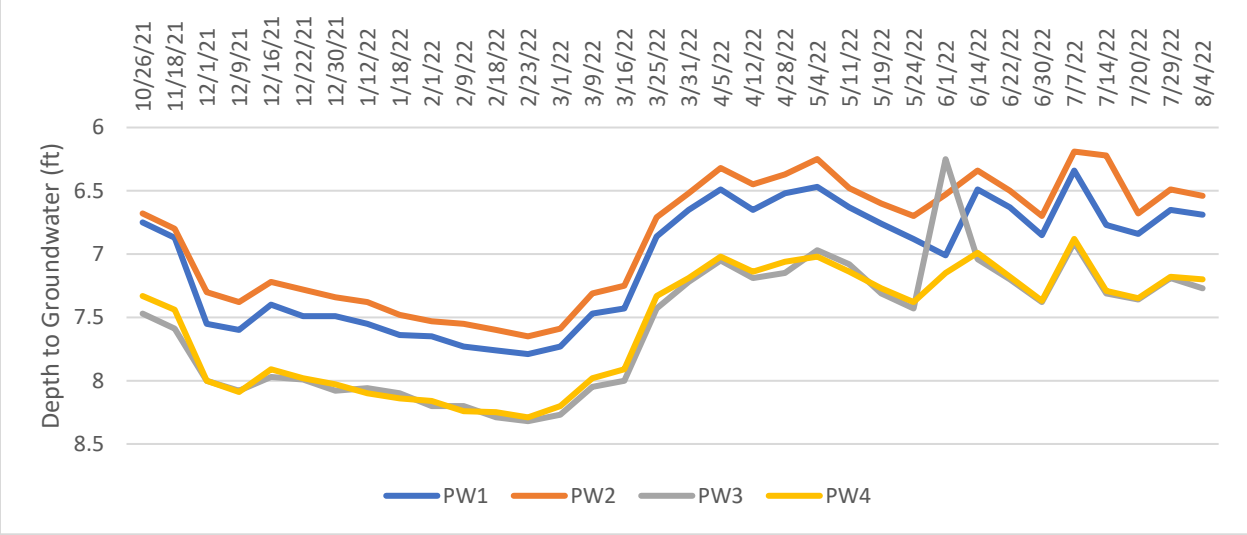

Figure 3. Summary of groundwater elevation data collected throughout the study period.

The subsurface lithology of the study area was found to consist of fine flowing sands. This is supported by historical geological studies of the area which show the subject location to be constructed on over 300+ ft of glacial drift that infilled a pre-glacial gorge. These sand and gravel deposits have been historically shown to support groundwater movements of 0.5-0.9 ft/day while other sources indicate the potential for movements over 1 ft/day. The heterogeneous nature of glacial drift makes it likely that hydraulic conductivities vary significantly over the site and, potentially, even within the project area. Regardless, groundwater flow through the project area appeared to be significant and likely resulted in a near constant influx of contaminants from up-gradient source zones which have been documented. This influx of contamination should be carefully considered when evaluating the results of this study.

Limited inorganic groundwater chemistry parameters were collected during the study period. pH values of the groundwater prior to injection activities were found to be circumneutral, ranging from approximately 6.6 to 7.5. Following the treatment injection, pH values in PW-1 climbed significantly to values exceeding 12. This was due to the inclusion of calcium peroxide as part of the initial treatment to provide a baseline level of dissolved oxygen while the oxygen generation system was configured. The pH spike was significantly less pronounced in PW-2 and non-existent in PW-4. Other inorganic chemistry parameters monitored throughout the study included total dissolved solids (TDS), conductivity, oxidation-reduction potential (ORP), dissolved oxygen (DO), calcium ions, and inorganic fluoride. Results from these parameters are discussed later in this report if/when pertinent.

<u>For the purposes of this study and the interpretation of the findings, the following considerations have been made regarding each monitoring well:</u>

- PW-1: Approximate center of treatment area. Representative of groundwater that is "mid-treatment" given the influx of PFAS contamination from upgradient sources.
- PW-2: Side-gradient to treatment. Periodic influxes of oxygen into this well seem to occur which maintain low-levels of biological activity.
- PW-3: Down-gradient of treatment area. Representative of "best case" for treatment of water as it fluxes out of the treatment area surrounding PW-1. Being down-gradient of the treatment area, this well has less influences caused by the inflow of contaminants due to upgradient injection of treatment chemistry.
- PW-4: Side-gradient to up-gradient of treatment area. PW-4 has typically had the lowest dissolved oxygen concentrations of any monitoring point as it has the lowest potential to be influenced by the oxygen generation system implemented. This well has low biological activity and is representative of treatment occurring without the support of microbial biomass. The lack of pH fluctuation and dissolved oxygen spike in PW-4 is further support that this area received minimal treatment and is more indicative of background conditions.

Summary of November 2021 Injection

On November 18th, 2021, ORIN began preparations for chemical injection activities by arriving on site, staging the injection equipment, and laying out injection locations. A tailgate health and safety meeting was held to discuss potential site hazards between ORIN, WI ANG, Onsite Environmental, and Covanta personnel prior to commencement of chemical injection. ORIN commenced injection activities at approximately 9:36 AM. Approximately 200 gallons of 12.8% BAM combined with 1% calcium peroxide, and PFAS Degrading Bacteria solution treatment chemistry was allocated for each of the 17 DPT injection points.

On the first two injection points, ORIN utilized its' side injection rods to deliver the treatment chemistry to the subsurface. However, ORIN changed to drop out tips after experiencing flowing sands that clogged the side injection rods. The drop out tips were able to remain closed while drilling to depth. Throughout the injection ORIN experienced minor daylighting to the surface through pathways between the ground

and the injection rods. In the northern portion of the injection area, daylighting occurred where treatment chemistry found a way to the surface through preferential pathways. When daylighting was observed, injection pressures and flow rates were decreased. If this did not stop the daylighting, the remaining volume was redistributed in an adjacent injection point.

ORIN employed the use of a vacuum extraction truck simultaneous to injection activities. The vac truck was on site on the 18th for half of the day. During that time the primary wells that were targeted via vacuum extraction were PW-1, PW-2, and PW-3. The maximum capacity for vacuum extraction was limited to 1,500 gallons due to the storage tank ORIN provided for offloading. Around mid-day, the vac truck operator notified ORIN that the truck was close to the capacity limit. Covanta and ORIN offloaded approximately 1,400 gallons of groundwater that had been extracted. At that moment ORIN determined to end vacuum extraction processes and the vac truck was escorted offsite. The extracted groundwater was treated with additional BAM and then disposed of by the WI ANG.

ORIN was able to observe treatment chemistry in each of the four wells on site. This observation was made by visual evidence via mounding of treatment chemistry in the well, via sight-glass from vac truck, or by lowering a bailer into the well for confirmation.

Injection activities were completed on November 19th at approximately 12:58PM. Following completion of the injection activities, ORIN and Onsite installed six electrodes for the oxygen generation system. After the electrodes were installed, all equipment, refuse, and subcontractors demobilized from the site. A total of 3,400 gallons of treatment chemistry was injected.

Results: Dissolved Oxygen

Dissolved oxygen (DO) concentrations were monitored weekly using a peristaltic pump, HDPE tubing, a Hanna Instruments Multi-meter, and a flow-through cell. Baseline concentrations of DO in the treatment area primarily reflected an aerobic to microaerobic environment, with concentrations ranging from 0.35 to 1.59 mg/L in the days before the injection of treatment products. It is known from previous studies that the microbes utilized to degrade PFAS only perform in sufficiently aerobic environments. These environments can either have high dissolved oxygen concentrations or a constant supply of lower DO levels to facilitate microbial respiration.

In previous field studies our teams have utilized both chemical oxidants and physical aeration to provide sufficient oxygen. However, given the long-term nature of this study and subsurface conditions, the use of a different system was selected. Our teams have previously utilized an oxygen generation system at several hydrocarbon remediation sites in western Canada to provide sustained oxygen for aerobic microbes. This system utilizes square wave direct current between 10 and 16 volts to hydrolyze water in situ and on soil particles, generating oxygen. At this particular site, six iron electrodes were utilized in banks of three placed opposite to one another around PW-1. The electrooxidation control unit was placed in Building 430 and cables were placed subsurface to provide power to each electrode.

A two-week gap between the injection of remedial products and the installation of the oxygen generation was anticipated. To ensure sufficient dissolved oxygen concentrations and to avoid the loss of injected microbial biomass, calcium peroxide was co-injected with the BAM and microbes to provide an initial boost of oxygen while long-term solutions were implemented.

Following the initial injection, which included chemical oxidants, a substantial spike in DO was observed in PW-1 and PW-2 and a smaller response was observed in PW-3. PW-4 saw only a marginal increase in DO concentrations, indicating that potentially less treatment had reached this area. DO in all wells began to rapidly decline in all wells in the weeks that followed. However, DO concentrations stabilized in PW-1 between 2 and 3 weeks post-treatment when the oxygen generation system was implemented. Over the duration of the study the DO in the treatment area was largely stable between 2 and 5 mg/L. Notably, PW-4 consistently had the lowest DO values that were often below those required to maintain biological activity (Fixed Earth recommends >2 mg/L DO as a minimum). Figure 4 provides a visual summary of field collected DO data.

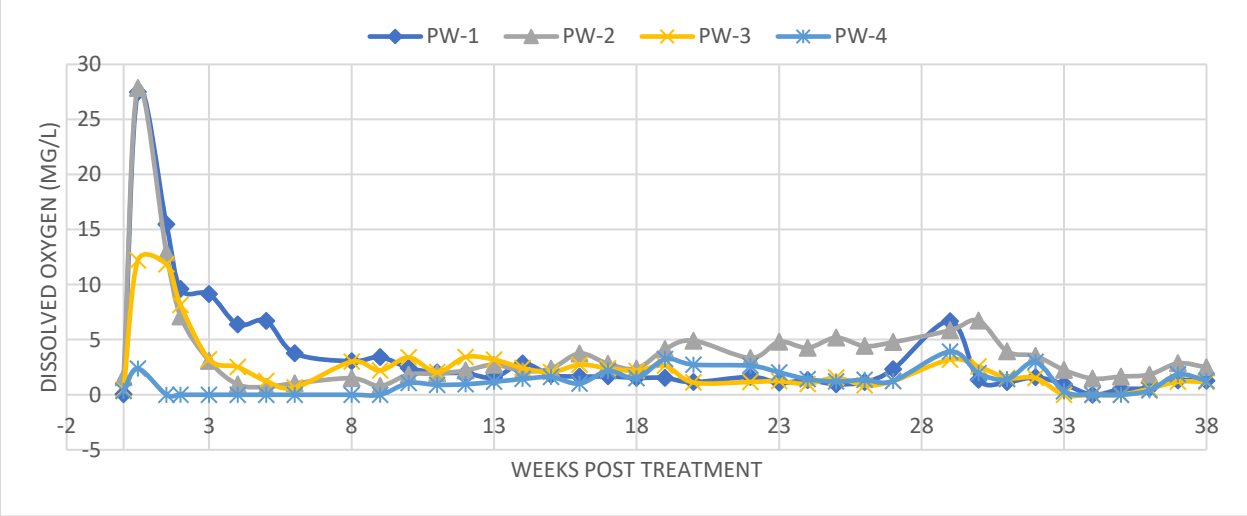

Figure 4. Summary of dissolved oxygen field monitoring data. Reference Appendix A for numerical data.

Results: PFAS Monitoring

Groundwater samples were collected from each monitoring well prior to injection to establish baseline conditions, and at 1, 3, 5, and 10 weeks following injection. After this time samples were collected monthly for the remainder of the study. All samples were analyzed via EPA Method 537.

Figure 5 provides a visual summary of PFAS removals from the wells as a percentage compared to baseline concentrations. A few key observations and interpretations can be made from this data when taken into consideration with the groundwater elevation data and dissolved oxygen data:

- All wells observed a significant (>80%) reduction in total PFAS concentrations one week following injection.
- PW-1 and PW-3 maintained >90% total PFAS removals through 10 weeks. This may be related to winter conditions in which minimal flushing of PFAS from the vadose zone was occurring. Any PFAS being transported into the treatment area is at a rate where biological activity was able to keep pace with the flux, maintaining low concentrations despite a constant inflow of contaminants.
- Rebound was observed within 2 weeks at PW-2 and PW-4 and this trend continued for several weeks. Both wells are considered side gradient to the treatment and inferred groundwater directional flow. Additionally, these wells quickly returned to near anaerobic conditions that are not conducive to microbial PFAS degradation and likely had minimal to no biological activity aside from the periodic influx of oxygen.
- Between 10 and 14 weeks spring freshet occurred resulting in a rapid rise in groundwater levels. During this time, PFAS concentrations in three of four wells increased. This may be due to flushing of PFAS from the vadose zone, increased groundwater speeds, and/or the rise of the groundwater table into more contaminated lithology.
- PFAS concentrations once again declined in PW-1 and PW-3 in the sampling events following freshet, suggesting that the conditions had sufficiently stabilized for biological activity to "catch-up." However, in areas where dissolved oxygen concentrations were less conducive to biological activity, namely PW-2 and PW-4, PFAS concentrations continued to climb and remain unpredictable.
- At 23 weeks post-injection, a significant spike in PFAS concentrations was observed in PW-4, with smaller spikes observed in PW-2 and PW-3. This is most likely attributed to the first significant rainfall event of the season on April 22 and caused groundwater elevations to reach their approximate maximum. PFAS concentrations in PW-4 returned to their pre-treatment baseline levels following this event and continued to fluctuate unpredictably.
- In areas where biological activity was supported, such as PW-3, PFAS concentrations once again declined following the week 23 spike. This pattern of PFAS removals following a spike in concentrations is reflective of a biological system returning to homeostasis and is difficult to attribute to sorption by BAM alone as sorption-based systems seldom see a decline following contaminant breakthrough. A closer examination of PW-3 monitoring data is presented in Figure 6.
- PW-3 maintained a 90+ percent reduction in total PFAS concentrations despite the inflow of contaminants from upgradient at ~1 ft/day and vadose zone flushing caused by spring freshet and rainfall events. This is in stark contrast to PW-4 where the biological system was non-functional due to lack of oxygen where very significant swings in total PFAS concentrations could be observed, suggesting that the BAM had reached its sorption capacity.
- Dissolved oxygen concentrations in PW-2 remained unpredictable at best, resulting in periods where PFAS concentrations would climb and others where they would decline.

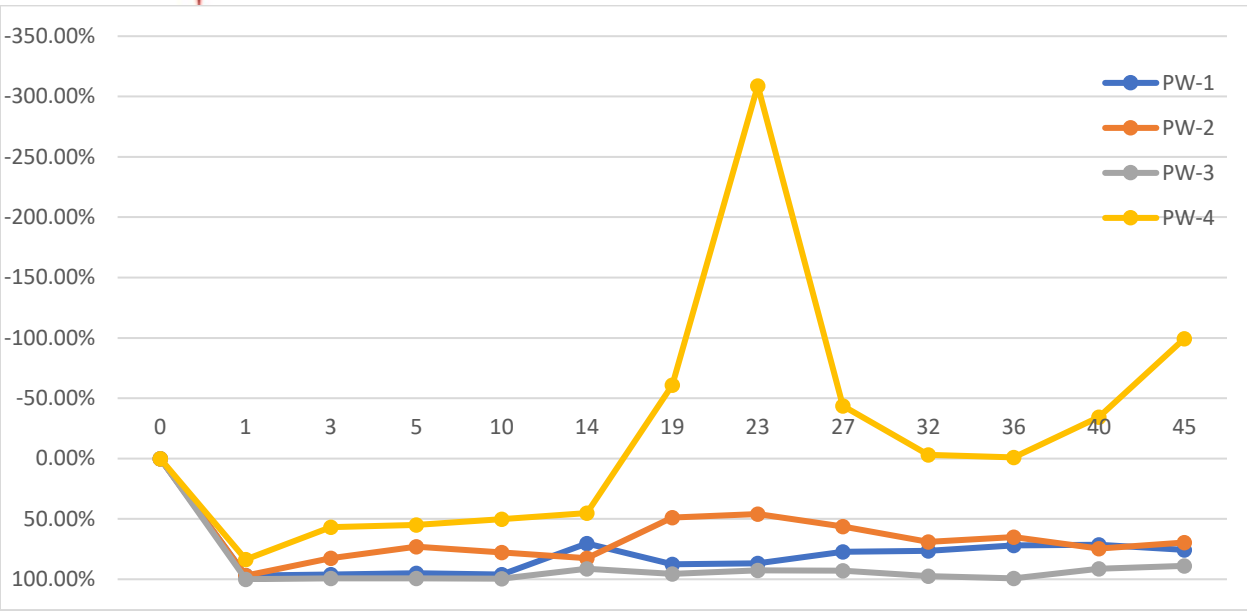

Figure 5. Summary of Total PFAS concentrations as a percentage of baseline concentrations.

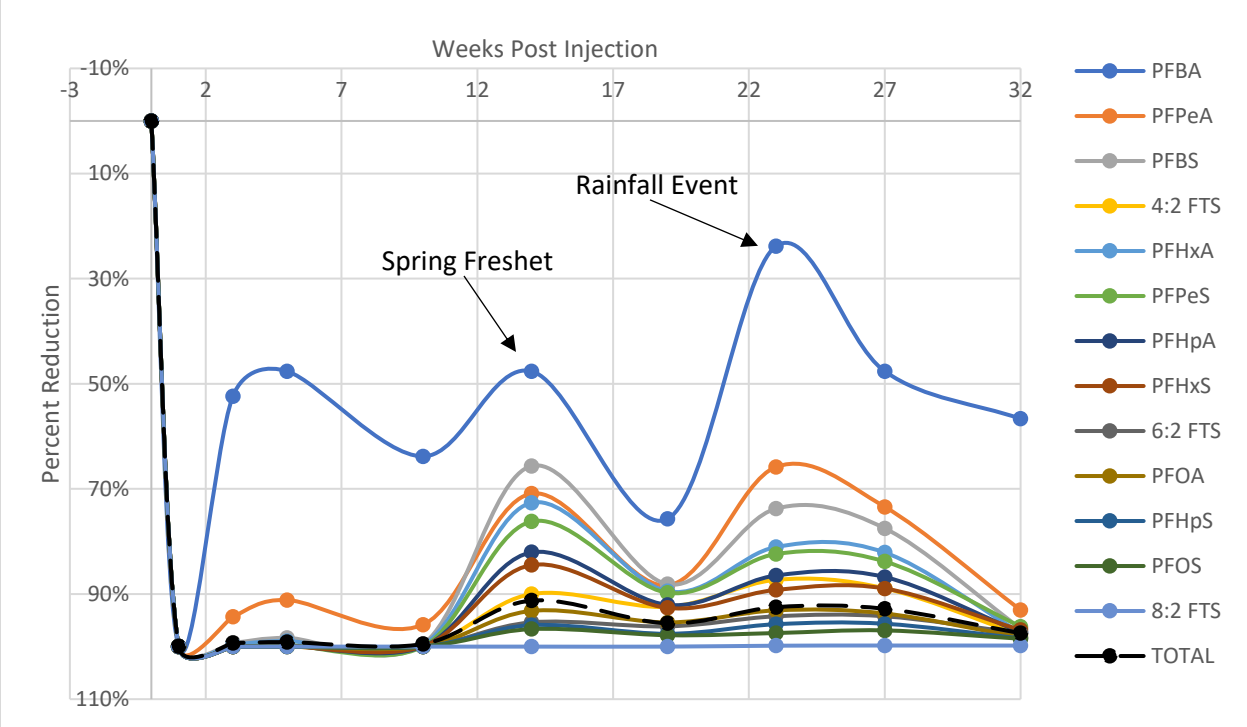

Figure 6. PW-3 PFAS monitoring data as a percentage compared to baseline.

Numerical presentations of all PFAS monitoring data can be found in Appendix A.

Results: Total Organic/Adsorbable Fluorine

Total Organic/Adsorbable Fluorine (TAF) was selected as a method to supplement analysis through EPA 537. The TAF method, in principle, should account for all fluorinated compounds in a water sample larger than 3-4 carbons in length. While there is no EPA standard method finalized to measure TAF, this is typically achieved by passing a water sample through a solid phase extraction (SPE) cartridge containing a sorbent, most often charcoal, with a known baseline fluorine content. The fluorinated compounds sorb to the SPE cartridge which is then incinerated and the resulting gas analyzed via combustion ion chromatography or similar methods. This method provides a quantitative measurement of all fluoride in a water sample that is part of an organic molecule. As a result, this method can be used to estimate precursor concentrations in a PFAS impacted water sample that are longer than 3 carbons in length. Previous research studies conducted by Fixed Earth using open-scan mass spectroscopy did not detect any fluorinated compounds that were 1-2 carbons in size. For the purposes of this study, TAF was utilized to ensure that carbon-fluorine cleavage and PFAS destruction was truly occurring and that molecules such as PFOS were not simply being transformed into a molecule that was undetectable via EPA 537 but potentially just as toxic.

A point of clarification is required regarding the methodology and the naming convention of the method(s). When these methods were first introduced, many labs advertised the method as “Total Organic Fluorine.” However, as the methods were utilized and their limitations better understood, such as the inability to measure ultra short-chain PFAS, many in the industry started referring to these methods as “Total Adsorbable Fluorine” to account for the fact that they only measured compounds which would adhere to activated charcoal. Although the methodology has not changed, the name used to describe these methods has evolved as our understanding of them increases. Given the similarity of the methods employed throughout the study, the results from all phases of the project should be directly comparable.

To interpret the results of this study, one must consider not only the TAF result independently, but also in consideration of the PFAS concentrations at the monitoring wells. In general, if PFAS concentrations decline significantly from baseline, but TAF concentrations fail to drop significantly it would be indicative of PFAS compounds simply being transformed by the biology into a form that is not visible to EPA Method 537. However, if both total PFAS concentrations and TAF drop significantly compared to baseline it is indicative of carbon-fluorine bond cleavage.

Figure 7 provides a visual overview of the TAF concentrations measured during the study period.

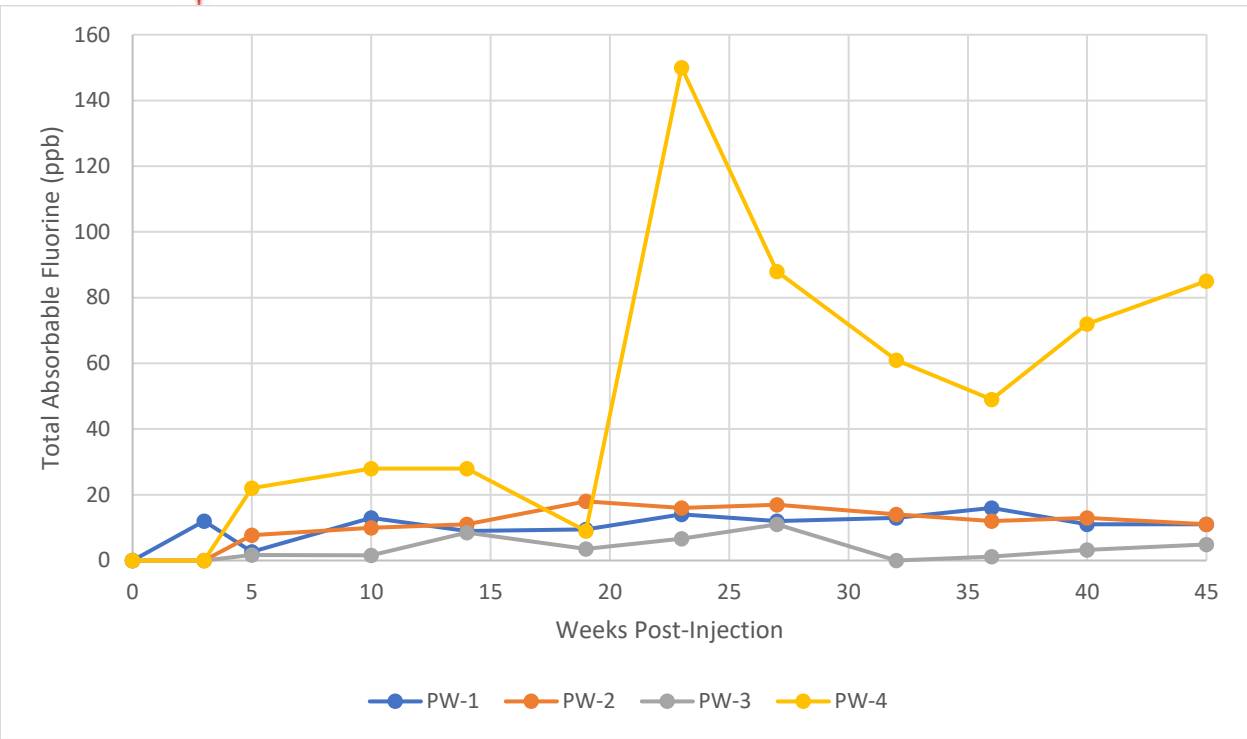

Figure 7. Total Adsorbable Fluorine concentrations within the study area.

It is noted that lab results indicate a baseline TAF concentration of 0 ppb. Given a total PFAS concentration of 35 ppb one would expect a TAF concentration of approximately 22.6 assuming a PFAS to TAF conversion rate of 0.64. To determine the conversion for PFAS to TAF, multiply by the percentage of a PFAS molecule that is made up of fluorine. For example, the TAF conversion rate for PFOS is 0.64 (as PFOS is comprised of 64% fluorine) multiplied by the PFOS concentration. A conversion rate of approximately 60% holds up for most PFAS species as an estimate. As such, the non-detect results observed at week 0 should be considerably higher. With this knowledge, a baseline concentration of 22.6 ppb assumed for the sake of data interpretation although TAF concentrations were likely much higher given the potential for precursor compounds not analyzed for in EPA 537. Overall, it is noted that TAF concentrations strongly correlate with total PFAS concentrations and leads us to trust the TAF data overall despite the unusual readings early in the study. However, as TAF is a method still being developed and refined by most commercial labs, the data should not be solely relied upon and only used in consideration with other supporting data.

This data is in support of the hypothesis that the carbon-fluorine bond is being broken and that PFAS removals are not simply due to partial defluorination or transformation. Similarly, we see a disconnect between TAF and total PFAS concentrations in PW-4 between the time of injection and freshet. During this time total PFAS concentrations remained at 50% reduced while TAF climbed to near the assumed baseline concentration. This would indicate that the lack of biological activity in PW-4 results in minimal defluorination.

Results: Potential Fluorite Formation

At approximately 10 weeks post-injection the groundwater in PW-1 was noted to be cloudy and contained a white precipitate. These precipitates were analyzed under a microscope to determine their nature. A portion of the precipitate was noted to be comprised of angular crystals.

Our team, using geochemical conditions similar to those found at the site (elevated pH and calcium ions causing calcium hydroxide to form, presence of peroxide, and presence of fluoride ions) were able to grow similar crystals, as shown in Figure 8. Notably, these crystals could not be produced unless fluoride was present in the geochemical mixture, implying the formation of a fluoride-based mineral.

When the pH of these solutions was adjusted and allowed to fall to circumneutral it was found that the square crystals dissipated. This matches field observations in which the cubic crystals and precipitates dissipated as the pH in PW-1 became less alkaline.

It is proposed that the observed precipitates are likely a divalent cation-fluoride mineral of some type, although more analysis beyond the scope of this study would be required to confirm or refute this hypothesis. The formation of fluoride minerals readily occurs at environmental pH and is a major hinderance in achieving a fluoride mass balance when studying PFAS degradation. These studies would need to be conducted at significantly acidic conditions, such as pH 4 or less, where these minerals do not form.

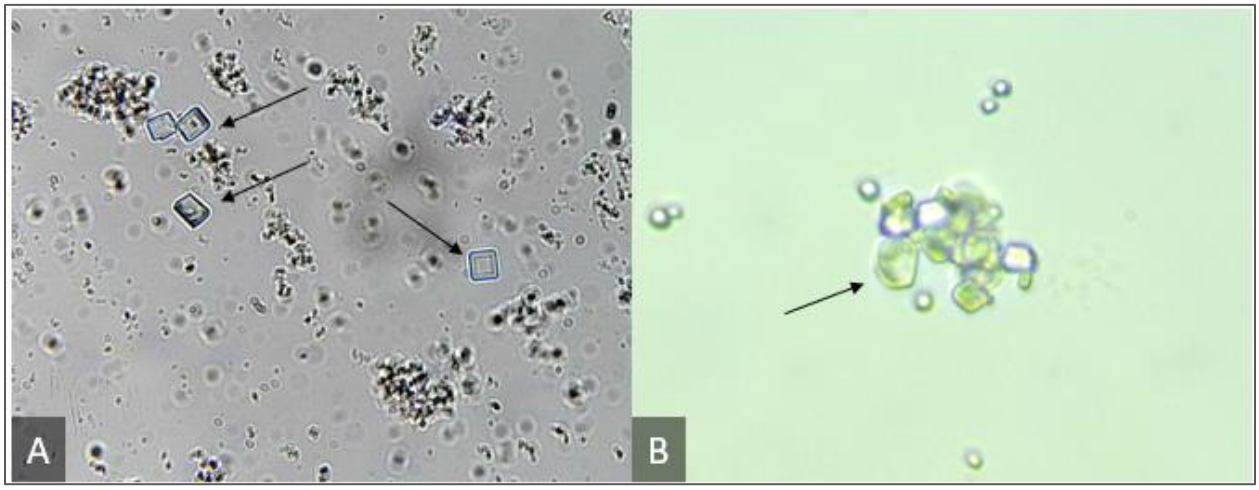

Figure 8. Lab grown putative fluorite crystals (A) and field observed precipitates (B).

Summary and Closure

The in-situ remediation study completed between October 2021 and November 2022 provided an excellent opportunity to evaluate the potential of biological PFAS remediation paired with BAM and oxygen generation chemistry over a variety of seasons and groundwater conditions. This dataset presents

a large and complex study in which multiple measured parameters must be considered together in order to arrive at findings and conclusions that are sound.

We present our overall findings and supporting facts as:

- PW-1 was representative of early stages in PFAS bioremediation when it is completed in an open system.
    - Groundwater flux through the base has been previously described as up to 1 ft/day. The significant groundwater gradient and sandy soils found in the treatment block support groundwater movements of a similar speed.
    - When spring resulted in an influx of PFAS, the system seemed able to respond to this influx and it declined in subsequent sampling events.
    - There are other PFAS source zones known up-gradient of this treatment block. As a result, it is probable that contaminant mass continued to flow into the treatment block and prevented PFAS concentrations from dropping as far as is possible in this well, as was demonstrated in the tote study (a closed system).
    - The failure of dissolved oxygen concentrations to climb significantly despite the constant generation of oxygen implies the consumption of DO. This was most likely by biomass consuming PFAS in the treatment block.
    - Putative fluorite crystals were observed in PW-1 suggesting sufficient fluoride being generated to cause precipitation.
    - TAF dropped significantly from the estimated baseline and remained low throughout the study implying the cleavage of the carbon-fluorine bond.

- PW-2 is representative of a system where the biology only functions marginally.
    - The location of this well is cross-gradient from the treatment block.
    - Dissolved oxygen concentrations rapidly declined after the injection and were, for significant periods, below the suggested concentrations to maintain biological activity and were elevated at other times.
    - PFAS concentrations in this area declined compared to baseline, but failed to perform to the same extent as more aerobic portions of the treatment block, such as PW-1 and PW-3.

- PW-3 is the best representation of biological PFAS treatment because it is more isolated from upstream effects.
    - Groundwater flow indicates that PW-3 is downgradient of the treatment block.
    - Being downgradient of the treatment block allows additional time for biological action against PFAS, resulting in larger reductions than those observed in PW-1.

  - Dissolved oxygen levels in PW-3 were generally lower than those in PW-1 suggesting that oxygen was consumed as groundwater flows from PW-1 towards PW-3, this is most likely through biological oxygen demand as the biomass consumed PFAS.
  - TAF in PW-3 was the lowest at the majority of sampling events and approaching zero at times.
  - Two PFAS influx events occurred at week 14 and week 23. In both cases the system was able to respond and PFAS concentrations reduced following the spike as the biology "caught up" and consumed the additional PFAS molecules and returned to homeostasis.
  - Dissolved oxygen concentrations often declined during PFAS influx events that would suggest an uptick in biological oxygen demand.

- PW-4 failed to support biological activity and should be treated as if BAM alone was used to treat it.
  - Groundwater flow in the area would place this well at cross-gradient from the treatment block.
  - Dissolved oxygen concentrations in this well remained low throughout the study and were generally below the threshold needed for biological activity.
  - Initially PFAS declined but began to rebound almost immediately.
  - No spike in pH or dissolved oxygen was observed immediately following the injection suggesting that little treatment fluid reached this area.
  - Total PFAS and TAF became decoupled for a portion of the study which was not observed at any other treatment point.
  - Large and unpredictable spikes in PFAS/TAF occurred during the study with no decline below baseline after the spikes.

When evaluating the degradation of PFOA and PFOS in comparison to current drinking water standards we are considering system performance during different periods of groundwater flux. During winter months when groundwater flux was reduced and little to no vadose zone flushing was occurring, we observed PFOA and PFOS concentrations well within current Wisconsin regulatory thresholds (NR 809), Wisconsin Department of Health Services (DHS) recommended groundwater standards, and Federal guidance. For context the Wisconsin DHS recommends that combined PFOA and PFOS concentrations be at 20 ppt or less for groundwater. On February 1, 2022, PW-3 had measured concentrations of 2.1 ppt PFOS and non-detectable PFOA. The system was showing similar performance in the sampling events prior to February 1, 2022. However, during freshet and during periods of higher groundwater flux and vadose zone flushing both PFOA and PFOS climbed over the regulatory thresholds. This, we believe, is not a weakness in the system performance, but rather highlights the key importance of treating larger source areas simultaneously to avoid perpetual flux of new contaminants into areas that have already been remediated. We also cite the 2021 tote study in a closed system as a demonstration that the combined treatment using BAM, microbes, and aeration is able to achieve the desired concentrations of PFOA and PFOS despite starting with extremely high amounts of both substances.

Overall, it is our opinion that this study was successful in demonstrating the field potential for the biological degradation of PFAS with enhancement through the use of BAM and an oxygen generation system. While there is significant potential for further research and development of this technology, we believe that many remaining concerns can be alleviated through this body of work as well as other public studies of these technologies.

ORIN Technologies and Fixed Earth appreciate the opportunity to provide our expertise in the field of environmental remediation for both the Wisconsin Air National Guard and the Dane County Regional Airport. ORIN and Fixed Earth look forward to future opportunities to aid in the issue of PFAS remediation.

Appendices:

A – Data Tables

B – Summary of Tote Study

C – February 2021 Microbe Report

ORIN Technologies

Appendix A: Pilot Well Analytical Results

PW-1														
Analyte ng/l	PW-1 10/26/21	PW-1 11/29/21	PW-1 12/16/21	PW-1 12/30/21	PW-1 2/1/22	PW-1 3/1/22	PW-1 4/5/22	PW-1 5/4/22	PW-1 6/1/22	PW-1 7/7/22	PW-1 8/4/22	PW-1 9/1/22	PW-1 10/6/22	PW-1 11/3/22
PFBA	350.0	150.0	280.0	250.0	290.0	190.00	330.0	330.0	250.0	190.0	200.0	200.0	230.0	210.0
PFPeA	1,500.0	300.0	450.0	580.0	430.0	470.00	930.0	1,100.0	680.0	550.0	640.0	660.0	660.0	600.0
PFBS	610.0	5.9	6.2	7.1	12.0	160.00	47.0	97.0	200.0	190.0	210.0	180.0	180.0	190.0
4:2 FTS	50.0	0.0	0.7	0.99	1.1	13.00	4.4	5.4	11.0	13.0	18.0	18.0	15.0	14.0
PFHxA	1,600.0	77.0	110.0	140.0	93.0	500.00	390.0	450.0	480.0	470.0	560.0	560.0	520.0	480.0
PFPeS	930.0	0.0	5.8	7.5	12.0	200.00	46.0	100.0	240.0	230.0	220.0	210.0	200.0	210.0
HFPO-DA	0.0	0.0						1.7						
PFHpA	510.0	14.0	18.0	26.0	19.0	190.00	91.0	99.0	130.0	150.0	180.0	190.0	150.0	160.0
PFHxS	6,900.0	29.0	43.0	43.0	86.0	1,800.00	280.0	450.0	1,400.0	1,500.0	1,400.0	1,600.0	1,200.0	1,200.0
6:2 FTS	3,500.0	290.0	260.0	460.0	140.0	820.00	1,200.0	990.0	1,000.0	710.0	1,000.0	670.0	840.0	890.0
PFOA	1,500.0	7.8	8.4	11.0	23.0	470.00	91.0	96.0	290.0	340.0	380.0	430.0	370.0	300.0
PFHpS	310.0	0.0	1.1	0.98	2.4	76.00	7.6	12.0	47.0	67.0	53.0	86.0	81.0	59.0
PFNA	73.0	0.0	0.65	0.54	0.99	27.00	3.6	5.8	14.0	16.0	19.0	19.0	13.0	12.0
PFOSA	18.0	6.0	1.7	3.0	0.61	0.51	9.0	6.1	1.3	1.9	0.0	1.7		3.8
PFOS	14,000.0	100.0	79.0	94.0	150.0	4,400.00	560.0	540.0	2,500.0	3,000.0	4,000.0	4,100.0	3,200.0	3,000.0
PFDA	7.3	0.0		0.53		2.80	1.3	1.0	1.4	2.6	0.0	2.8		1.7
8:2 FTS	670.0	17.0	6.1	11.0	8.2	260.00	59.0	36.0	110.0	250.0	270.0	390.0	270.0	240.0
PFNS	0.0					27.00	0.54		12.0			1.9	20.0	20.0
NMeFOSAA	0.0					2.10				1.3		3.1		
PFUnA	0.0									0.61				
PFDS	0.0													
10:2 FTS	0.0					0.92				1.6				
TOTAL	**32,528**	**996.7**	**1,270.7**	**1,636**	**1,268**	**9,609.33**	**4,050.4**	**4,320.0**	**7,366.7**	**7,684.0**	**9,150.0**	**9,322.5**	**7,949.0**	**7,590.5**
Calcium (mg/L)	110.00	NS	190.00	210.00	17.00	87	13.0	14	97.0	100.00	100.00	100.0	110.0	110.0
Fluoride (mg/L)	0.17	NS	0.03	0.04	0.04	0.10	0.050	0.06	0.12	0.13	0.14	0.18	0.2	0.18
Adsorbable Organic Fluorine (ug/L)	0.00	NS	0.00	12.00	2.70	13	9.0	9.5	14.0	12.00	13.00	16.0	11.0	11.0

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PW-2														
Analyte ng/l	PW-2 10/26/21	PW-2 11/29/21	PW-2 12/16/21	PW-2 12/30/21	PW-2 2/1/22	PW-2 3/1/22	PW-2 4/5/22	PW-2 5/4/22	PW-2 6/1/22	PW-2 7/7/22	PW-2 8/4/22	PW-2 9/1/22	PW-2 10/6/22	PW-2 11/3/22
PFBA	390.0	140.0	170.0	240.0	180.00	260.00	230.0	490.0	290.0	220.0	240.0	250.0	210.0	220.0
PFPeA	1,300.0	250.0	390.0	540.0	370.00	370.00	670.0	1,200.0	860.0	590.0	650.0	540.0	550.0	510.0
PFBS	560.0	57.0	180.0	250.0	170.00	160.00	240.0	230.0	150.0	190.0	230.0	160.0	150.0	150.0
4:2 FTS	20.0		14.0	20.0	13.00	8.20	7.0		1.6	2.0	0.0	1.3	0.97	1.3
PFHxA	1,400.0	120.0	360.0	570.0	390.00	410.00	760.0	1,300.0	960.0	490.0	730.0	580.0	700.0	700.0
PFPeS	880.0	32.0	190.0	300.0	210.00	39.00	310.0	430.0	270.0	270.0	320.0	210.0	210.0	200.0
HFPO-DA	0.0								1.2					
PFHpA	380.0	17.0	76.0	140.0	110.00	99.00	200.0	400.0	260.0	140.0	170.0	120.0	130.0	140.0
PFHxS	7,000.0	130.0	1,300.0	1,800.0	1,500.00	410.00	2,500.0	3,300.0	3,300.0	2,200.0	2,300.0	1,500.0	1,500.0	1,500.0
6:2 FTS	1,400.0	41.0	550.0	940.0	670.00	380.00	520.0	410.0	200.0	150.0	95.0	62.0	67.0	68.0
PFOA	560.0	21.0	200.0	240.0	180.00	140.00	240.0	250.0	180.0	160.0	170.0	120.0	120.0	88.0
PFHpS	410.0		41.0	64.0	51.00	39.00	120.0	110.0	98.0	91.0	54.0	66.0	110.0	42.0
PFNA	170.0		13.0	20.0	22.00	23.00	81.0	88.0	64.0	40.0	52.0	32.0	44.0	41.0
PFOSA	8.2				0.81	0.88	1.9		2.5	2.0	0.0	1.4	1.7	
PFOS	16,000.0	120.0	1,800.0	3,100.0	2,900.00	3,000.00	9,700.0	8,300.0	6,700.0	4,900.0	5,700.0	4,100.0	5,500.0	5,200.0
PFDA	0.0			0.74	0.62		0.96		0.91	1.3		0.49	0.7	0.45
8:2 FTS	150.0	0.0	36.0	46.0	50.00	38.00	59.0	42.0	29.0	27.0	14.0	13.0	10.0	9.5
PFNS	0.0			6.4		5.40	27.0	18.0	21.0				35.0	11.0
NMeFOSAA	0.0								1.6					
PFUnA	0.0									0.68				
PFDS	0.0									0.8				
10:2 FTS	0.0									0.98				
TOTAL	**30,628.2**	**928.0**	**5,320.0**	**8,277.1**	**6,817.43**	**5,382.48**	**15,666.9**	**16,568.0**	**13,389.8**	**9,475.8**	**10,725.0**	**7,756.2**	**9,339.4**	**8,881.3**
Calcium (mg/L)	99.00	NS	99.00	99.00	99.00	91.00	90.0	75.0	85.0	84.00	91.00	92.0	91.0	88.0
Fluoride (mg/L)	0.20	NS	0.15	0.17	0.17	0.16	0.16	0.2	0.21	0.26	0.21	0.25	0.24	0.23
Adsorbable Organic Fluorine (ug/L)	0.00	NS	0.00	7.70	10.00	11.00	18.0	16.0	17.0	14.00	12.00	13.0	11.0	10.0

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PW-3														
Analyte ng/l	PW-3 10/26/21	PW-3 11/29/21	PW-3 12/16/21	PW-3 12/30/21	PW-3 2/1/22	PW-3 3/1/22	PW-3 4/5/22	PW-3 5/4/22	PW-3 6/1/22	PW-3 7/7/22	PW-3 8/4/22	PW-3 9/1/22	PW-3 10/6/22	PW-3 11/3/22
PFBA	210.0	0.0	100.0	110.0	76.0	110.00	51.0	160.0	110.0	91.0	54.0	130.0	170.0	160.0
PFPeA	790.0		45.0	70.0	33.0	230.00	92.0	270.0	210.0	55.0	9.8	210.0	370.0	390.0
PFBS	320.0		2.3	5.3	1.5	110.00	38.0	84.0	72.0	11.0	1.9	88.0	140.0	160.0
4:2 FTS	37.0					3.70	2.8	4.7	4.1	0.83		2.7	0.9	3.5
PFHxA	950.0		4.1	10.0	3.0	260.00	100.0	180.0	170.0	25.0	4.6	180.0	340.0	330.0
PFPeS	420.0			1.1		100.00	43.0	74.0	68.0	16.0	3.0	93.0	150.0	170.0
HFPO-DA	0.0							1.6	0.8					
PFHpA	340.0			0.52		61.00	27.0	46.0	45.0	9.3	2.0	42.0	90.0	100.0
PFHxS	3,800.0		0.84	2.4	1.7	590.00	280.0	410.0	420.0	120.0	31.0	460.0	670.0	780.0
6:2 FTS	2,600.0					120.00	100.0	150.0	150.0	66.0	12.0	110.0	56.0	130.0
PFOA	1,000.0			0.49		68.00	46.0	69.0	63.0	18.0	4.7	55.0	69.0	93.0
PFHpS	230.0					9.40	5.6	9.8	10.0	3.6	1.0	14.0	18.0	18.0
PFNA	44.0					2.70	2.2	3.0	3.0	1.3		3.0	2.4	3.1
PFOSA	0.0	11.0				0.70			0.66	1.8	0.64	1.2	0.76	
PFOS	12,000.0		2.8	1.7	2.1	400.00	260.0	310.0	370.0	180.0	62.0	630.0	550.0	630.0
PFDA	6.3	7.5												
8:2 FTS	860.0							1.5	1.8	1.7		8.5	13.0	12.0
PFNS	0.0					2.70							1.6	0.93
NMeFOSAA	5.7													
PFUnA	0.0													
PFDS	0.0													
10:2 FTS	0.0									1.3				
TOTAL	**23,613.0**	**18.5**	**155.0**	**201.5**	**117.3**	**2,068.20**	**1,047.6**	**1,773.6**	**1,698.4**	**601.8**	**186.6**	**2,027.4**	**2,641.7**	**2,980.5**
Calcium (mg/L)	100.00	NS	3.40	81.00	160.00	58.00	75.0	85.0	89.0	87.0	1.2	86.0	87.0	89.0
Fluoride (mg/L)	0.18	NS	0.05	0.05	0.05	0.06	0.09	0.13	0.11	0.1	0.11	0.13	0.14	0.14
Adsorbable Organic Fluorine (ug/L)	0.00	NS	0.00	1.70	1.60	8.50	3.5	6.6	11.0	ND	92.0	3.2	4.9	5.8

PW-4														
Analyte ng/l	PW-4 10/26/21	PW-4 11/29/21	PW-4 12/16/21	PW-4 12/30/21	PW-4 2/1/22	PW-4 3/1/22	PW-4 4/5/22	PW-4 5/4/22	PW-4 6/1/22	PW-4 7/7/22	PW-4 8/1/22	PW-4 9/1/22	PW-4 10/6/22	PW-4 11/3/22
PFBA	390.0	360.0	460.0	460.0	380.0	390.00	980.0	2,000.0	1,100.0	710.0	660.0	1,100.0	1,100.0	680.0
PFPeA	1,500.0	670.0	1,600.0	1,400.0	1,400.0	1,400.00	4,800.0	8,300.0	4,900.0	3,700.0	3,200.0	4,900.0	4,100.0	2,400.0
PFBS	650.0	98.0	150.0	200.0	320.0	380.00	500.0	1,000.0	450.0	480.0	500.0	460.0	490.0	320.0
4:2 FTS	51.0	5.8	4.4	8.5	13.0	21.00	12.0	18.0	12.0	20.0	0.0	13.0	11.0	12.0
PFHxA	1,600.0	350.0	710.0	870.0	1,000.0	1,300.00	3,900.0	6,200.0	3,300.0	2,400.0	2,000.0	2,800.0	3,200.0	1,800.0
PFPeS	1,000.0	120.0	300.0	390.0	540.0	620.00	1,300.0	2,700.0	1,200.0	990.0	810.0	1,100.0	1,200.0	720.0
HFPO-DA	0.0													
PFHpA	510.0	85.0	140.0	220.0	280.0	320.00	950.0	1,700.0	880.0	590.0	490.0	760.0	770.0	510.0
PFHxS	8,500.0	1,200.0	3,200.0	3,500.0	4,700.0	4,900.00	22,000.0	48,000.0	19,000.0	11,000.0	7,400.0	14,000.0	15,000.0	9,000.0
6:2 FTS	3,100.0	710.0	2,200.0	2,300.0	2,600.0	2,200.00	4,800.0	21,000.0	3,700.0	3,700.0	5,100.0	3,700.0	9,100.0	9,400.0
PFOA	1,100.0	130.0	250.0	300.0	280.0	490.00	1,300.0	3,700.0	1,400.0	770.0	660.0	740.0	960.0	680.0
PFHpS	350.0	48.0	220.0	230.0	180.0	210.00	950.0	3,700.0	1,100.0	560.0	330.0	1,000.0	1,700.0	750.0
PFNA	93.0	13.0	50.0	43.0	38.0	46.00	290.0	820.0	290.0	130.0	110.0	270.0	320.0	260.0
PFOSA	88.0		7.9	11.0	41.0	29.00	69.0	120.0	94.0	90.0	91.0	78.0	160.0	83.0
PFOS	18,000.0	2,300.0	6,700.0	6,700.0	6,600.0	7,900.00	18,000.0	53,000.0	16,000.0	13,000.0	16,000.0	19,000.0	36,000.0	33,000.0
PFDA	6.6			4.5	6.5	5.60		19.0	9.1	7.5	0.0	9.9	8.5	8.9
8:2 FTS	350.0	36.0	98.0	130.0	180.0	220.00	150.0	170.0	160.0	290.0	300.0	180.0	150.0	310.0
PFNS	0.0		4.2		11.0		32.0	17.0	4.6	4.8			48.0	
NMeFOSAA	0.0			1.3	1.1	1.30								
PFUnA	0.0			0.65	1.6									
PFDS	0.0		8.3	9.1	34.0	5.60			15.0					
10:2 FTS	0.0				1.2	2.40		12.0	8.8	12.0				
TOTAL	**37,288.6**	**6,125.8**	**16,102.8**	**16,778.1**	**18,607.4**	**20,440.90**	**60,033.0**	**152,476.0**	**53,623.5**	**38,454.3**	**37,651.0**	**50,110.9**	**74,317.5**	**59,933.9**
Calcium (mg/L)	91.00	NS	48.00	53.00	62.0	69	68.0	61.0	65.0	66.0	66.0	65.0	69	77
Fluoride (mg/L)	0.20	NS	0.16	0.16	0.16	0.15	0.12	0.14	0.14	0.16	0.15	0.17	0.17	0.16
Adsorbable Organic Fluorine (ug/L)	0.00	NS	0.00	22.00	28.0	28	9.0	150.0	88.0	61.0	49.0	72.0	85	81

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PW-1	PW-1 10/26/21	PW-1 11/29/21	PW-1 12/16/21	PW-1 12/30/21	PW-1 2/1/22	PW-1 3/1/22	PW-1 4/5/22	PW-1 5/4/22	PW-1 6/1/22	PW-1 7/7/22	PW-1 8/4/22	PW-1 9/1/22	PW-1 10/6/22	PW-1 11/3/22
Depth to Water (ftbgs)	6.75	-	7.4	7.49	7.65	7.73	6.49	6.47	7.01	6.34	6.69	6.68	6.8	7.09
pH	6.88	12.69	12.65	12.61	12.03	7.5	11.17	10.95	7.6	7.3	6.82	6.74	7.02	6.69
ORP	21	-34	-18	-57.1	36.5	-116.7	67.5	38.1	-152.1	-109	-97.7	-87.9	-56.9	-24.5
DO (mg/L)	0.06	15.48	6.38	3.77	2.44	2.84	1.53	1.08	2.3	1.58	0.6	1.74	1.69	2.82
Conductivity (mS/cm)	0.983	4.167	4.292	3.861	1.871	0.857	0.787	0.53	0.93	0.851	0.863	0.821	0.797	0.912
Total Disolved Solids (ppm)	491	2641	2137	1931	943	427	394	259	461	428	432	410	399	456
Temp. (°C)	15.49	13.88	13.36	12.72	11.36	10.77	9.28	10.14	12.16	14.16	15.21	15.2	15.94	15.95

PW-2	PW-2 10/26/21	PW-2 11/29/21	PW-2 12/16/21	PW-2 12/30/21	PW-2 2/1/22	PW-2 3/1/22	PW-2 4/5/22	PW-2 5/4/22	PW-2 5/31/22	PW-2 7/7/22	PW-2 8/4/22	PW-2 9/1/22	PW-2 10/6/22	PW-2 11/3/22
Depth to Water (ftbgs)	6.68	-	7.22	7.34	7.53	7.59	6.32	6.25	6.53	6.19	6.54	6.52	6.68	6.92
pH	6.94	9.6	7.5	7.16	6.82	6.8	7.18	7.19	7.13	7.19	6.84	6.91	7.02	7.16
ORP	22.8	96.1	128.1	180.6	164.2	134.8	158.6	160.4	128.4	88.3	164.4	131.5	163.7	51.5
DO (mg/L)	1.51	12.91	0.94	1.04	1.83	2.15	4.14	4.8	4.77	3.52	1.83	2.34	1.88	3.48
Conductivity (mS/cm)	0.805	0.892	0.894	0.84	0.871	0.863	0.818	0.668	0.714	0.74	0.738	0.732	0.677	0.7
Total Disolved Solids (ppm)	403	446	446	420	436	432	409	334	357	370	369	366	339	350
Temp. (°C)	16.98	14.48	13.24	13.09	11.91	11.62	9.25	10.75	13.53	15.08	16.09	16.59	17.01	16.69

PW-3	PW-3 10/26/21	PW-3 11/29/21	PW-3 12/16/21	PW-3 12/30/21	PW-3 2/1/22	PW-3 3/1/22	PW-3 4/5/22	PW-3 5/4/22	PW-3 6/1/22	PW-3 7/7/22	PW-3 8/4/22	PW-3 9/1/22	PW-3 10/6/22	PW-3 11/3/22
Depth to Water (ftbgs)	7.47	-	7.97	8.08	8.2	8.27	7.05	6.97	6.25	6.91	7.27	7.2	7.42	7.61
pH	7.03	12.3	12.52	12.55	12.44	8.4	7.91	7.01	7.22	7.38	7.47	7.13	7	7.03
ORP	42.9	-7.6	-1.4	-46.4	-30.5	35.7	95.8	-126.5	-87.1	56.2	126.9	158.8	168.6	53.4
DO (mg/L)	0.24	11.85	2.52	0.65	3.38	2.42	2.63	1.24	1.24	1.52	0.55	1.58	1.53	2.78
Conductivity (mS/cm)	0.85	2.531	2.734	2.542	3.496	0.671	0.738	0.807	0.801	0.754	0.735	0.701	0.682	0.74
Total Disolved Solids (ppm)	425	1269	1214	1280	1777	336	369	403	401	378	367	351	341	370
Temp. (°C)	15.64	14.22	12.29	11.45	10.11	9.28	8.2	9.65	11.64	13.91	15.35	16.76	16.78	16.18

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PW-4	PW-4 10/26/21	PW-4 11/29/21	PW-4 12/16/21	PW-4 12/30/21	PW-4 2/1/22	PW-4 3/1/22	PW-4 4/5/22	PW-4 5/4/22	PW-4 6/1/22	PW-4 7/7/22	PW-4 8/4/22	PW-4 9/1/22	PW-4 10/6/22	PW-4 11/3/22
Depth to Water (ftbgs)	7.33	-	7.91	8.03	8.16	8.2	7.02	7.02	7.15	6.88	7.2	7.22	7.31	7.61
pH	7.09	7.25	7.39	7.24	6.8	6.74	7.12	6.99	7.15	7.2	6.83	6.91	7.04	6.94
ORP	57	194	153.4	62.2	-18.5	-50.4	19.8	59.2	42.4	-5.7	17.1	102.5	173.6	46.1
DO (mg/L)	0.71	<0.5	<0.5	<0.5	1.05	1.43	3.23	2.03	1.24	2.97	0.39	1.63	1.68	3.2
Conductivity (mS/cm)	0.768	0.474	0.493	0.498	0.635	0.687	0.659	0.611	0.594	0.68	0.588	0.595	0.584	0.672
Total Disolved Solids (ppm)	384	237	247	249	318	344	329	305	297	340	294	298	292	336
Temp. (°C)	15.74	13.94	11.84	12.04	10.8	9.89	7.49	9.26	11.27	15.21	14.47	15.69	15.89	15.55

Appendix B: Ex-situ Tote Study Summary

Subject: Treatment Report for the Wisconsin Air National Guard in Madison, WI.

The following is a summary of the work completed to date by ORIN Technologies, LLC. (ORIN) for the Wisconsin Air National Guard (WI ANG) at Truax Field in Madison, Wisconsin.

On May 28th, 2021, ORIN began preparations for chemical treatment of four totes containing PFAS impacted water. Activities included obtaining pre-treatment samples, measuring pH, dissolved oxygen, and treatment dosing. Treatment remedies consisted of BAM Ultra and PFAS Degrading Bacteria. Totes 1, 3, & 4 received 180 lbs. of BAM Ultra, approximately 1 lb. of calcium peroxide, and a 1-liter solution of PFAS Degrading Bacteria. Tote 2 received 1 lb. of calcium peroxide and a 1-liter solution of PFAS Degrading Bacteria, no BAM Ultra was added to Tote 2. Following the addition of the treatment remedies, the totes were mixed using a paddle mixer. Each tote had an aerator to supply oxygen for sustaining the PFAS Degrading Bacteria.

Over the following weeks ORIN made observations on the totes collecting pH and dissolved oxygen data. Analytical samples were collected eight times over a span of 35 weeks. Prior to collection each tote was mixed with the designated paddle mixer. After the Week 1 sampling event, Tote 1 displayed 58.13% total reduction, Tote 2 – 51.93% reduction, Tote 3 – 97.53% reduction, and Tote 4 – 97.22% reduction. After 35 weeks of reaction time, Tote 1 measured 99.53% reduction, Tote 2 – 95.05% reduction, Tote 3 – 99.94% reduction, and Tote 4 – 99.97% reduction in total PFAS via EPA Method 537. The reduction of PFAS in Tote 2 proves that PFAS Degrading Bacteria can be isolated and cultured to breakdown PFAS compounds. The combination of BAM and PFAS Degrading Bacteria were able to reduce Tote 1 by over 99% which began with greater than 33ppm total PFAS.

	TOTE 1 - BAM Ultra with PFAS Degrading Bacteria								
Analyte ng/l	Baseline Mix	Week 1	Percent Change	Week 5	Percent Change	Week 15	Percent Change	Week 35	Percent Change
PFBA	356,000.0	333,000.0	6.46%	309,000.0	13.20%	44,000.0	87.64%	35,000.00	90.17%
PFPeA	1,090,000.0	1,130,000.0	-3.67%	1,050,000.0	3.67%	140,000.0	87.16%	78,000.00	92.84%
4:2 FTS	46,100.0	41,700.0	9.54%	34,200.0	25.81%	590.0	98.72%	86.00	99.81%
PFHxA	1,170,000.0	978,000.0	16.41%	834,000.0	28.72%	40,000.0	96.58%	11,000.00	99.06%
PFPeS	54,700.0	28,000.0	48.81%	10,300.0	81.17%	720.0	98.68%	130.00	99.76%
PFHpA	19,800.0	25,900.0	-30.81%	17,900.0	9.60%	210.0	98.94%	41.00	99.79%
ADONA	0.0	0.0		0.0					
PFHxS	345,000.0	67,800.0	80.35%	12,500.0	96.38%	170.0	99.95%	28.00	99.99%
6:2 FTS	27,100,000.0	9,460,000.0	65.09%	1,450,000.0	94.65%	1,800.0	99.99%	580.00	100.00%
PFOA	50,700.0	14,600.0	71.20%	0.0	100.00%	10.0	99.98%		100.00%
PFHpS	43,200.0	11,600.0	73.15%	0.0	100.00%		100.00%		100.00%
PFNA	0.0	41,400.0		7,460.0		21.0		15.00	
PFOSA	0.0	0.0		0.0				5.80	
PFOS	2,790,000.0	630,000.0	77.42%	6,870.0	99.75%	16.0	100.00%	22.00	100.00%
8:2 FTS	32,100.0	7,700.0	76.01%	0.0	100.00%		100.00%		100.00%
MeFOSAA	15,800.0	0.0	100.00%	0.0	100.00%		100.00%		100.00%
10:2 FTS	29,500.0	14,500.0	50.85%	0.0	100.00%		100.00%		100.00%
TOTAL	**33,142,900.0**	**12,784,200.0**	**61.43%**	**3,732,230.0**	**88.74%**	**227,537.0**	**99.31%**	**124,907.80**	**99.62%**

	TOTE 2 - PFAS Degrading Bacteria								
Analyte ng/l	Baseline Mix	Week 1	Percent Change	Week 5	Percent Change	Week 15	Percent Change	Week 35	Percent Change
PFBA	160,000.0	192,000.0	-20.00%	179,000.0	-11.88%	32,000.0	80.00%	31,000.00	80.63%
PFPeA	733,000.0	795,000.0	-8.46%	685,000.0	6.55%	150,000.0	79.54%	110,000.00	84.99%
4:2 FTS	12,400.0	10,300.0	16.94%	9,520.0	23.23%	2,800.0	77.42%	2,400.00	80.65%
PFHxA	251,000.0	308,000.0	-22.71%	304,000.0	-21.12%	66,000.0	73.71%	51,000.00	79.68%
PFPeS	17,200.0	0.0	100.00%	0.0	100.00%	2,100.0	87.79%	500.00	97.09%
PFHpA	11,100.0	9,260.0	16.58%	6,790.0	38.83%	1,500.0	86.49%	410.00	96.31%
ADONA	0.0	0.0		0.0					
PFHxS	166,000.0	19,600.0	88.19%	7,540.0	95.46%	1,800.0	98.92%	330.00	99.80%
6:2 FTS	7,280,000.0	1,500,000.0	79.40%	4,460,000.0	38.74%	500,000.0	93.13%	72,000.00	99.01%
PFOA	18,600.0	0.0	100.00%	0.0	100.00%	550.0	97.04%	66.00	99.65%
PFHpS	12,700.0	0.0	100.00%	0.0	100.00%	61.0	99.52%	18.00	99.86%
PFNA	0.0	309,000.0		228,000.0		24,000.0		5,000.00	
PFOSA	7,510.0	0.0	100.00%	0.0	100.00%	490.0	93.48%	200.00	97.34%
PFOS	1,060,000.0	185,000.0	82.55%	155,000.0	85.38%	27,000.0	97.45%	9,900.00	99.07%
8:2 FTS	18,800.0	0.0	100.00%	8,860.0	52.87%	1,800.0	90.43%	1,400.00	92.55%
MeFOSAA	0.0	0.0		0.0					
10:2 FTS	0.0	10,600.0		0.0					
TOTAL	**9,748,310.0**	**3,338,760.0**	**65.75%**	**6,043,710.0**	**38.00%**	**810,101.0**	**91.69%**	**284,224.0**	**97.08%**

	TOTE 3 - BAM Ultra with PFAS Degrading Bacteria								
Analyte ng/l	Baseline Mix	Week 1	Percent Change	Week 5	Percent Change	Week 15	Percent Change	Week 35	Percent Change
PFBA	18,900.0	0.0	100.00%	0.0	100.00%	19.0	99.90%	36.00	99.81%
PFPeA	47,600.0	0.0	100.00%	0.0	100.00%	6.0	99.99%	7.10	99.99%
4:2 FTS	0.0	0.0		0.0					
PFHxA	50,100.0	0.0	100.00%	0.0	100.00%	8.0	99.98%	9.10	99.98%
PFPeS	0.0	0.0		0.0		0.47			
PFHpA	0.0	0.0		0.0		11.0			
ADONA	0.0	0.0		0.0					
PFHxS	26,900.0	0.0	100.00%	0.0	100.00%	4.6	99.98%		100.00%
6:2 FTS	1,130,000.0	0.0	100.00%	12,300.0	98.91%	380.0	99.97%	230.00	99.98%
PFOA	0.0	0.0		0.0		1.0			
PFHpS	0.0	0.0		0.0					
PFNA	0.0	19,300.0		8,800.0		460.0		330.00	
PFOSA	0.0	0.0		0.0		0.43		11.00	
PFOS	73,800.0	0.0	100.00%	0.0	100.00%	31.0	99.96%	28.00	99.96%
8:2 FTS	0.0	0.0		0.0		2.2			
MeFOSAA	0.0	0.0		0.0					
10:2 FTS	0.0	0.0		0.0					
TOTAL	**1,347,300.0**	**19,300.0**	**98.57%**	**21,100.0**	**98.43%**	**923.7**	**99.93%**	**651.20**	**99.952%**

	TOTE 4 - BAM Ultra with PFAS Degrading Bacteria								
Analyte ng/l	Baseline Mix	Week 1	Percent Change	Week 5	Percent Change	Week 15	Percent Change	Week 35	Percent Change
PFBA	122,000.0	79,600.0	34.75%	60,000.0	50.82%	2,700.0	97.79%	1,700.00	98.61%
PFPeA	220,000.0	47,700.0	78.32%	20,900.0	90.50%	490.0	99.78%	150.00	99.93%
4:2 FTS	9,420.0	0.0	100.00%	0.0	100.00%	0.7	99.99%		100.00%
PFHxA	199,000.0	0.0	100.00%	0.0	100.00%	35.0	99.98%	14.00	99.99%
PFPeS	12,600.0	0.0	100.00%	0.0	100.00%	0.86	99.99%		100.00%
PFHpA	8,100.0	0.0	100.00%	0.0	100.00%	2.2	99.97%		100.00%
ADONA	0.0	0.0		0.0					
PFHxS	85,100.0	0.0	100.00%	0.0	100.00%	4.3	99.99%		100.00%
6:2 FTS	5,260,000.0	18,200.0	99.65%	0.0	100.00%	340.0	99.99%		100.00%
PFOA	19,000.0	0.0	100.00%	0.0	100.00%	1.5	99.99%		100.00%
PFHpS	13,700.0	0.0	100.00%	0.0	100.00%	0.0	100.00%		100.00%
PFNA	0.0	0.0		0.0		12.0			
PFOSA	0.0	0.0		0.0		0.72		12.00	
PFOS	602,000.0	0.0	100.00%	0.0	100.00%	28.0	100.00%	6.60	99.999%
8:2 FTS	7,390.0	0.0	100.00%	0.0	100.00%	0.0	100.00%		100.00%
MeFOSAA	7,460.0	0.0	100.00%	0.0	100.00%	0.0	100.00%		100.00%
10:2 FTS	6,500.0	0.0	100.00%	0.0	100.00%	0.0	100.00%		100.00%
TOTAL	**6,572,270.0**	**145,500.0**	**97.79%**	**80,900.0**	**98.77%**	**3,615.3**	**99.94%**	**1,882.6**	**99.97%**

ORIN Technologies

Appendix C: February 2021 Microbe Report

February 7, 2021

ORIN Technologies LLC
405 Investment Court, Verona, WI, 53593
Attn: Larry Kinsman

RE: Interim Report – Acquisition and Validation of PFAS Degrading Microorganisms

Introduction and Background

Fixed Earth Innovations Ltd. (Fixed Earth) was retained by ORIN Technologies LLC (ORIN) to acquire microbes capable of degrading perfluoroalkyl substances (PFAS) from the Dane Country Airport located in Madison, WI (subject location) and has PFAS contamination present in media on site. The scope of this study included: Acquisition of PFAS degrading organisms, long-term storage of microbes, basic characterization of microbes, confirmation of C-F bond cleavage, desktop-scale validation of PFAS degradation, and testing microbe performance when exposed to ORIN's biochar products. This report is to serve as an interim update on work completed to-date and serves only to provide an overview. A detailed report will be prepared at the conclusion of the study and presented to ORIN for review.

Microbial Acquisition

ORIN provided samples containing soil, used biochar, and surface water from the subject location in mid-November 2020 to serve as source material in Fixed Earth's microbial acquisition method. Microbial acquisition commenced within 24 hours of sample receipt and was monitored in the days following to observe microbial growth. Microbial growth was observed after 48 hours and verified via transmitted light microscopy, as shown in Figure 1.

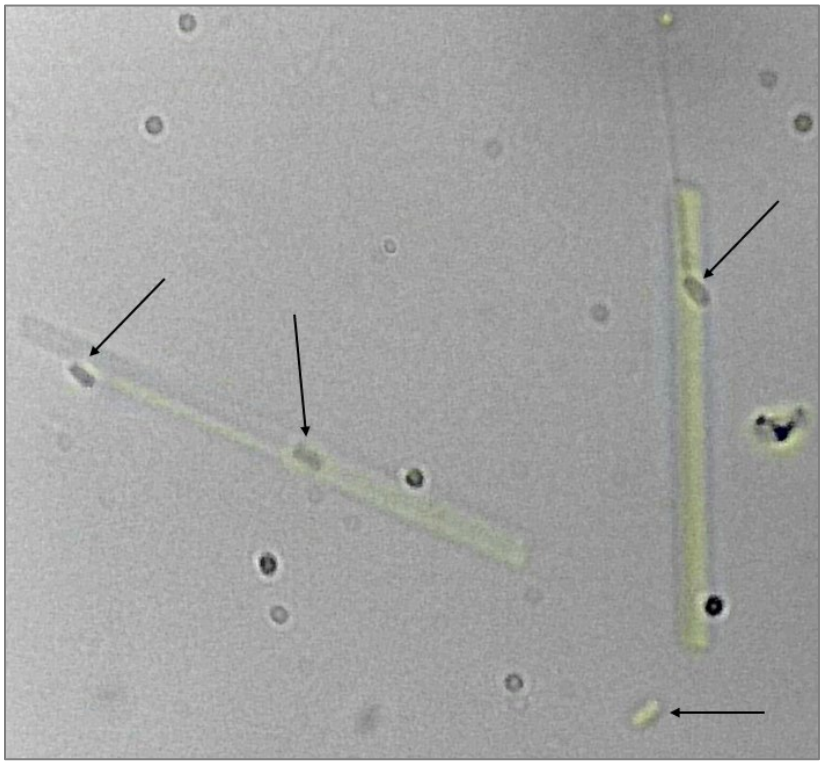

Figure 1. Initial Bacterial Growth from Airport Media Samples (Black Arrows) with PFAS Crystals. Taken at 1000x Magnification.

SASKATOON
#105 333 Nelson Road
Saskatoon, SK, S7S 1P2
Phone: 306.291.3264

FORT ST. JOHN
8615 100 Street
Fort St John, BC, V1J 3W7
Phone: 250.329.5207

A total of three distinct culturable bacteria with putative PFAS degradation abilities were acquired from site media and were identified as PFAS-US-WI-001, PFAS-US-WI-002, and PFAS-US-WI-003.

Notably, monitoring of sample growth showed a high degree of microbial diversity based on cell morphology. However, only three bacterial forms were found to be culturable on solid media. It is likely that a portion of the microbes present on the site are not culturable on standard microbiology media. Fixed Earth has undertaken preliminary studies to culture an increased diversity of organisms from environmental media and this work is expected to continue through 2021.

In the time following the provision of site media, Fixed Earth has also developed methods to isolate PFAS degrading fungal organisms which was further tested using site media. In addition to the three bacterial isolates above, a fungal organism was acquired and identified as PFAS-US-WI-004.

Freezer stocks of each organism was prepared in accordance with standard microbiological protocols. For bacterial isolates this consisted of 50% glycerol stocks stored at -20 °C. For fungal isolates this consisted of storing dry spores on granular charcoal at -20 °C. Freezer stocks were validated following one week of storage to ensure stock viability in storage. In Q2 2021 each stock will be split and stored in two distinct locations to prevent stock loss.

Microbe Characterization

Each microbe acquired from site media were characterized in a basic manner to provide sufficient identifying characteristics for each isolate so that their identity may be confirmed in the future as required. At this time no genetic identification of isolates to a genus or species level has occurred. Table 1 provides an overview of each isolate.

Table 1. Microbe Characterization

Name	Type	Colony Description	Metabolite Production	Microscopic Description
PFAS-US-WI-001	Bacteria	1-3 mm, Punctiform, Light Tan	Abundant Extra-Cellular Polymeric Substances, Polyhydroxyalkanoates	Gram Negative, Bacilli, Significant EPS
PFAS-US-WI-002	Bacteria	1-3 mm, Punctiform, Yellow, Slow Growing on LB	Trace Extra-Cellular Polymeric Substances	Gram Positive, Clusters of Cocci, Trace EPS
PFAS-US-WI-003	Bacteria	Large, Off-White, Undulate, Raised, Glassy, Rapid Growth on LB	Polyhydroxyalkanoates	Gram Negative, Diplobacilli, PHAs Evident, Motile
PFAS-US-WI-004	Bacteria	Fungal, Compact, Green Spores	Droplets of Secondary Metabolites Evident with Mature Spores	N/A

Photo plates of each bacterial isolate taken at 1000x magnification with oil-immersion can be found in Figure 2. Photos were taken following Gram staining.

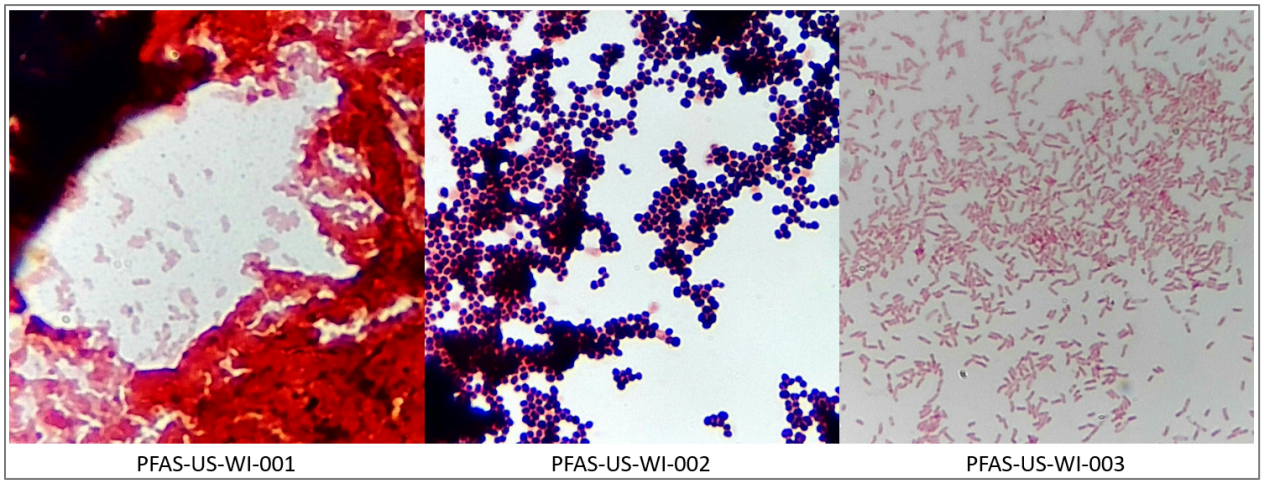

Figure 2. Photo Plates of Putative PFAS Degrading Bacterial Isolates. Taken at 1000x Magnification.

Carbon-Fluorine Bond Cleavage

A qualitative colourimetric test previously designed and validated by Fixed Earth to evaluate the ability of microbes to cleave the carbon-fluorine bond found in PFAS compounds was utilized to further characterize microorganisms.

In this test, an indicator dye is utilized which interacts with heavy metal ions to produce a distinct pink-red colour. Fluoride ions, formed from the metabolic breakdown of PFAS, interfere with the metal-dye complex, causing the colour to change to yellow. The only documented interference with this assay are high concentrations of phosphate in the growth media. Under controlled laboratory conditions, this interference can be easily avoided.

To complete this assay, microorganisms are grown on a specialized solid media containing fluorinated compounds for five to seven days. Following the growth period, a layer of the colourimetric reagent is poured over the bacterial colonies and allowed to solidify. A small quantity of sodium fluoride solution is added to an area free of microbes to serve as a positive control for fluoride. A positive result for fluoride formation is shown as a yellow halo surrounding microbial colonies.

Fungal organism PFAS-US-WI-004 was found to produce fluoride ions when grown in the presence of perfluoro compounds. Further detail can be observed in Figure 3.

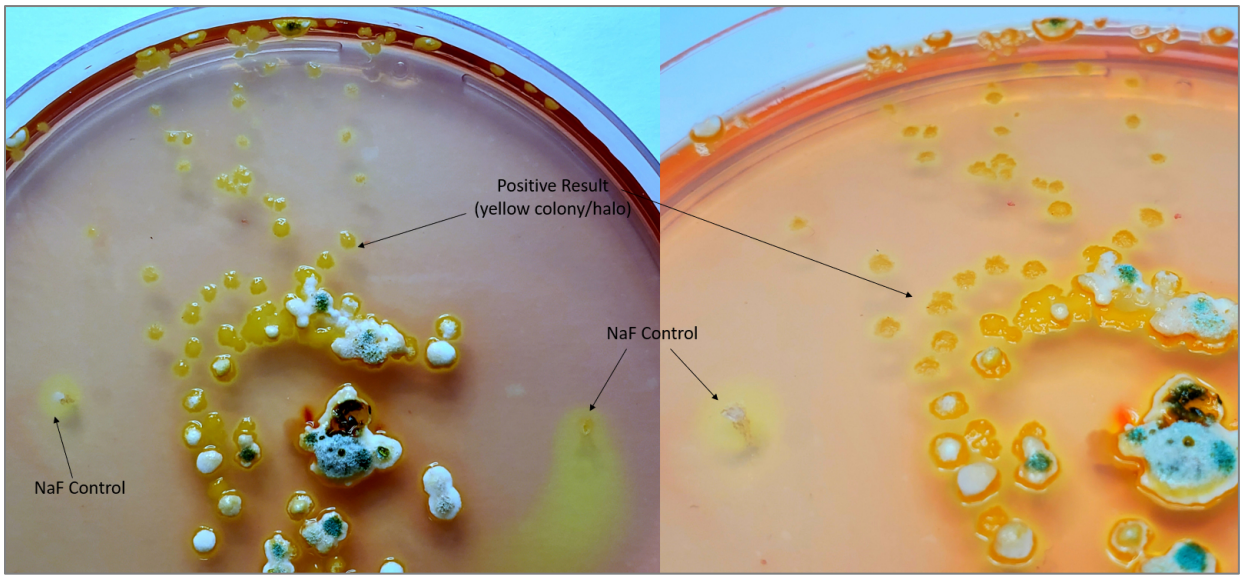

Figure 3. Fluoride Formation by Fungal Organism PFAS-US-WI-004.

Bacterial organisms PFAS-US-WI-001, 002, and 003 were each found to form fluoride when grown in the presence of perfluoro compounds. In particular, colonies of WI-001 and WI-002 were found to turn strongly yellow, with less conclusive results observed for WO-003. Further detail can be observed in Figure 4.

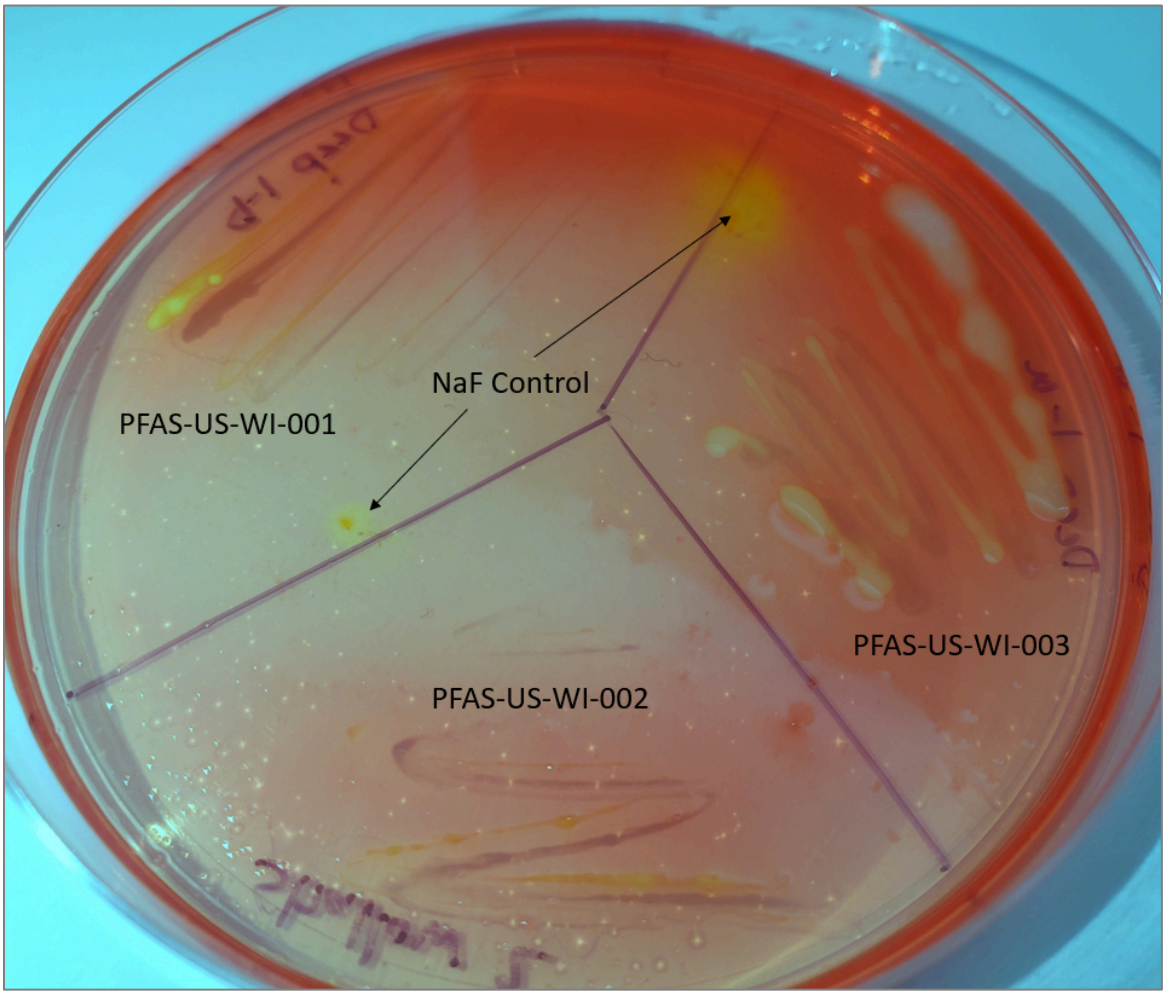

Figure 4. Fluoride Formation by Bacterial Organisms PFAS-US-WI-001, 002, and 003.

The test shown in Figure 4 was duplicated on a secondary petri plate to confirm or refute the observations. Similar results were obtained where WI-001 and WI-002 showed strong indications of fluoride formation while WI-003 was less conclusive.

BAM and Microbe Co-Culture

Microbes and BAM were added to a solution containing high concentrations of PFAS and maintained on a rotary shaker for a week to better understand possible interactions between BAM and PFAS degrading organisms. Water samples were collected following four and six days of co-culture and observed via light microscopy. Attention was paid to relative microbial abundance, formation of biofilms, biofilm proximity to biochar particles, etc.

After four days of culture early biofilm formation could be observed on a subset of biochar particles. Additionally, individual bacteria could be observed attached to small biochar particles which would serve as a precursor for larger biofilms. Photographs of biofilms and bacteria can be found in Figure 5.

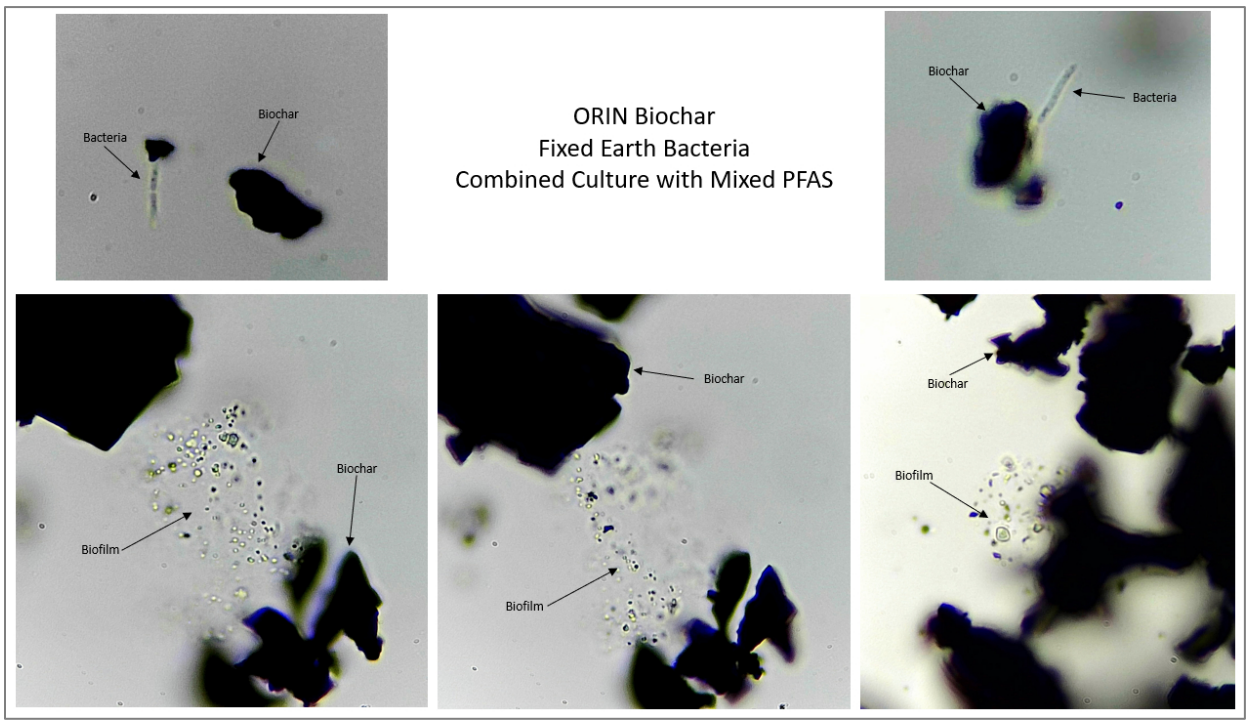

Figure 5. Four Day Biofilm Formation in PFAS, Biochar, and Microbe Co-Culture. Taken at 1000x Magnification.

At six days of co-culture, more complex and larger biofilms were noted to have formed. Additionally, biofilms could be observed on a larger percentage of biochar molecules.

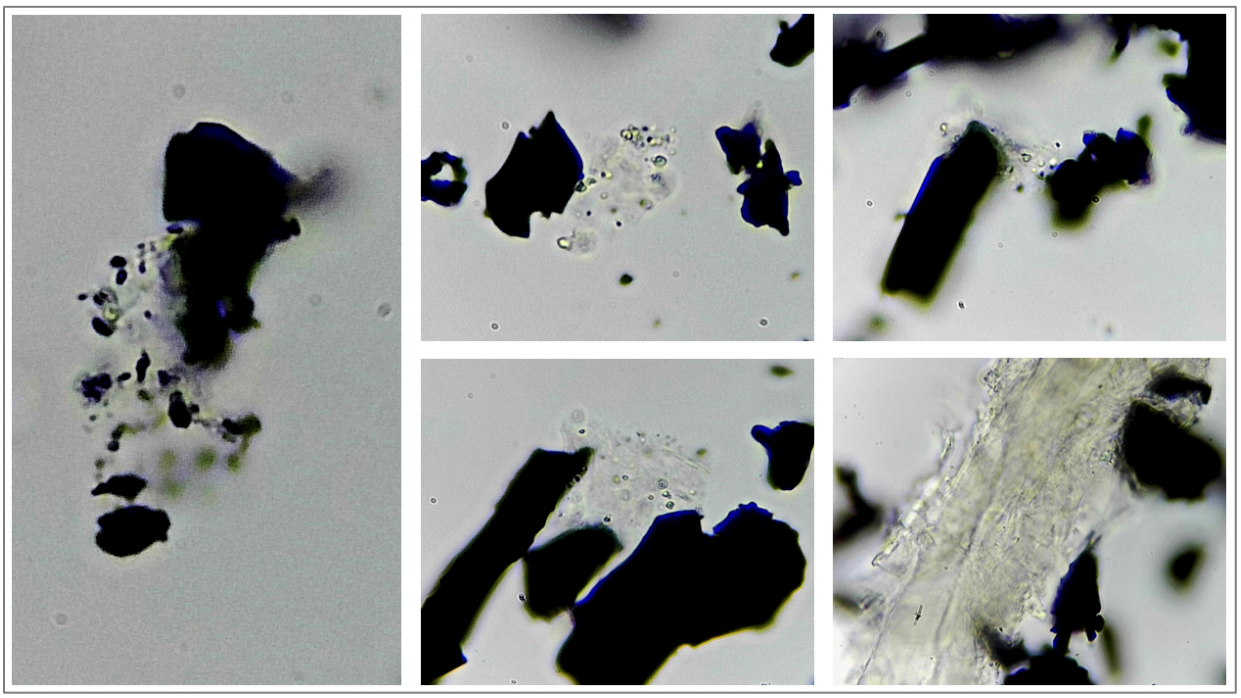

Figure 6. Six Day Biofilm Formation in PFAS, Biochar, and Microbe Co-Culture. Taken at 1000x Magnification.

Biofilms were generally found to attach to one side of the biochar molecules rather than coating them in entirety. It is unknown if the location of biofilms relative to the biochar molecule will impact the performance of either product and additional studies are warranted (see Work In-Progress). The co-culture of biochar and microbes is still active at the time of this interim report and additional results are pending.

Desktop Trial

A desktop trial was completed to provide analytical validation of the microbe's ability to degrade PFAS. Two three-litre bioreactors were prepared using 3.5 L Florence flasks washed in accordance with best practices and filled with 3 kg of sterilized tap water. Tap water was spiked with 2000 parts per trillion (ppt) of perfluorooctane sulfonate (PFOS). Analytical standards of PFOS purchased from Toronto Research Chemicals to ensure accuracy of measurements and purity of PFOS.

One bioreactor was sealed using a rubber stopper and remained untreated to serve as a control. Concentrated microbes were grown in PFAS-free culture to serve as inoculant for the second bioreactor. Microbes were titrated into the second bioreactor at a rate of 0.5 mL per liter of water. The second bioreactor was aerated to maintain aerobic conditions.

Samples were collected following one and two weeks of treatment and submitted to ALS Environmental for analysis of PFAS via EPA Method 537. When comparing control samples between sampling events, it was found that analytical noise prevented interpretation of data. It is likely that the utilized concentrations of PFOS are close to the method detection limit, reducing accuracy of measurements.

Samples have been resubmitted to Bureau Veritas Laboratory in Ontario, Canada for further analysis using methods with a higher sensitivity and reliability. Analytical results from the re-analysis of samples is pending and will be provided upon receipt.

Work In-Progress

As of the time of this writing, a number of experiments are planned or in-progress based on the initial workplan or adaptations proposed by ORIN and Fixed Earth based on previous observations.

A summary of planned work is as follows:

- Re-analysis of the first desktop trial results is ongoing and results are expected in February 2021.
- Microbe and biochar co-culture is ongoing and will be monitored;
- A second desktop trial is being designed in collaboration with ORIN to better understand the effects of utilizing mixed products on the retention and degradation of PFAS; and
- Preparation of a detailed report at the conclusion of the project.

Additional studies may be considered in collaboration with ORIN as the opportunities or concepts arise.

Closure

Please contact the undersigned at 250-329-5207 for any questions or concerns related to this letter. Thank you for the opportunity to take part in this project.

Prepared by:

Timothy Repas, MSc, PAg (BC)
President